REMARKS

This Amendment is presented in an effort to expedite and conclude the prosecution of the instant patent application. New claims 257-269 are directed to more specific embodiments of the claimed invention. Such claims specify that the growth factor is locally placed in the body of the human patient and that the growth factor comprises living stem cells harvested from bone marrow. Such claims are directed to the same invention previously claimed, but are more specific in some regards. No new matter is contained in the new claims. Basis for the new claims may be found at pages 45 and 46 of the specification where a growth factor, such as a cell, is placed in the body of the patient to grow new cardiac muscle and a new artery and at Examples 11-16 on pages 40-42 where living stem cells that are harvested from bone marrow are identified as stem cells that differentiate during morphogenesis. Injection of growth factors and localized administration thereof is disclosed at page 21, lines 4-10. In addition claims 238, 239, and 243 have been amended to depend upon claim 236 rather than cancelled claim 237. Claim 256 was cancelled and presented again as independent claim 264 because claim 256 previously depended upon a cancelled claim, and Applicant desired to require localized placement of a stem cell in a human patient.

Applicant also presents herewith further evidence and two recent decisions of the Court of Appeals for the Federal Circuit ("CAFC") for the Examiner's consideration. Such information and legal authority when considered with the accompanying remarks is believed to bear upon points raised by the Examiner during the Final Rejection. Applicant believes that such evidence and authority should result in simplifying, as well as expediting, the prosecution of the instant application.

Claim 254 [245] stands rejected under 35 U.S.C. §112, second paragraph, on the ground that the "multifactorial and non-specific" claim language is indefinite. Applicant understands that the Examiner intended to reject claim 245 in this rejection, rather than claim 254.

Prior to discussing this rejection, Applicant directs the attention of the Examiner to the recent *en banc* decision of the CAFC in Phillips v. AWH Corporation, 03-1269-1286, decided July 12, 2005. While the Phillips case involved patent claim infringement, Applicant believes that the principles and authorities expressed in this case are equally applicable for providing guidance to the Patent and Trademark Office (hereinafter "PTO") in determining the meaning of terms in the specification and claims of a pending patent application.

The Phillips decision indicated that the claims of a patent are generally given their ordinary and customary meaning in the art, citing the Vitronics v. Conceptronic, Inc., 90 F. 3d 1582 (Fed. Cir. 1996). Also cited was the Multiform Desiccants, Inc. v. Medzorn, Ltd. Decision, 133 F. 3d 473, 1477 (Fed. Cir. 1980) for the principle that claims should be read in the context of the patent. The Court in Phillips further observed that extrinsic evidence is less significant than the intrinsic record in determining the legally operative meaning of claim language, citing C.R. Bard, Inc. v. U.S. Surgical Corp., 388 F. 3d 858, 862 (Fed. Cir. 2000). The Court in Phillips also stated that dictionary evidence can be useful in claim interpretation, but that such evidence is less reliable than the patent specification and its prosecution history. Applicant thus submits that the Examiner should interpret the words "multifactorial" and "non-specific" in light of the specification as would be apparent to a person skilled in the medical art and thus give such words their ordinary meaning in the art to which the invention pertains. A different interpretation, such as that foisted by the Examiner, bottomed on non-contextual sources, places the term out of context and thus would not be entitled to the same weight of evidence in the interpretation of Applicant's disclosure by a skilled person in the medical art.

In the instant prosecution, the Examiner's position basically is supported by a lack of search results regarding these terms followed by a series of suppositions and speculations regarding the

meaning of these terms. Applicant believes that the Examiner's position amounts to no more than opinion because no objective evidence related to the medical art is presented. Moreover, the Examiner's prior position (that "multifactorial" describes a process) appears to be taken from a chemist's perspective, not from the perspective of one skilled in the medical art reading the instant specification. Had the Examiner viewed the term "factor" as a skilled medical person as opposed to a chemist, she would have then understood the term in the context of the medical art and would have raised no issue of indefiniteness. The meaning of the term "factor" is well known in the medical art, and one skilled in such art would have no difficulty understanding this term. Obviously, one understanding the medical term "factor" would also understand the term "multifactorial" to mean "more than one factor."

The Examiner is reminded that Applicant previously located and filed relevant search evidence in the Fifth Supplemental Information Disclosure Statement ("IDS") filed on October 21, 2004 (via fax) regarding the definitions of the terms. Apparently, the Examiner failed to locate Applicant's above-mentioned evidence in her search. In any event, the definitions of "multifactorial" and "non-specific" presented in the IDS provide confirming evidence that the disputed terms are known and used properly in Applicant's specification. Note further that the IDS identifies these term as adjectives.

Applicant conducted a search of the NIH Medical Dictionary and found the following definitions in Merriam Webster's Medline Plus Medical Dictionary (attached hereto as Exhibits A and B):

Factor:	***(noun)*** A substance that functions in or promotes the function of a particular physiological process or bodily system.

Multifactorial: ***(adjective)*** Having, involving, or produced by a variety of elements or causes.

Thus, the noun "factor," as used in Applicant's specification, means a substance, such as a cell, that promotes a particular physiological process, such as the growth of an artery. "Multifactorial" is an adjective used to denote a quality of a cell. A cell is deemed to be "multifactorial" when a variety (more than one) of elements (factors) promote the growth of an artery. Accordingly, there can be no doubt that the term "multifactorial" is used properly in the specification, and that its meaning would be clear to one skilled in the medical art. The above-mentioned definitions are consistent with Applicant's specification, with the materials furnished in the IDS, and with the use of this term by those skilled in the art, as further explained below.

Applicant further notes that the terms were understood by skilled persons in the art, i.e., by Drs. Heuser and Lorincz, in paragraph 10 of their Second Supplemental Declarations (of record). Additionally, an understanding of the questioned terms by workers skilled in the medical art consistent with the description in Applicant's specification and the definition of "multifactorial" in the IDS can be found in the 2003 publication of Strauer et al. entitled, "Stem Cell Therapy in Perspective" (hereinafter referred to as "Strauer 2003" and attached hereto as Exhibit C) and in the 2005 publication of Strauer et al. entitled, "Regeneration of Human Infarcted Heart Muscle by Intracoronary Autologous Bone Marrow Cell Transplantation in Chronic Coronary Artery Disease" (hereinafter referred to as "Strauer 2005" and attached hereto as Exhibit D). In this regard, note the reference to cardiac lesions (cardiac wounds or damage) as "multifactorial" in Strauer 2003 at page 7, paragraph 6. In Strauer 2005, Dr. Strauer states at page 1656, second column, third paragraph that, "The regenerative potential of bone-marrow-derived stem cells may be explained by any of four mechanisms." These four cell-biologic and molecular mechanisms are further described as

"factors" at page 1657, second column, second full paragraph. Therefore, it is clear to a skilled person in the medical art that Dr. Strauer and his co-authors identify the regenerative potential of bone marrow stem cells as being derived from at least four different mechanisms or characteristics of such cells. It follows that bone marrow stem cells can be appropriately styled as four-factor cells, i.e., multifactorial. This interpretation of said term is consistent with the disclosure of the instant application, with the definitions in above-mentioned Exhibits A and B, with the definitions submitted in the IDS, and with the above-mentioned understandings of Drs. Heuser and Lorincz. Thus, taken together, Strauer 2003 and Strauer 2005 confirm that another skilled group of medical experts possesses an understanding of "multifactorial" that is consistent with that of Applicant and the evidentiary materials discussed herein. Certainly, Applicant's above-mentioned evidence, when considered with the authoritative statements and tenets of Phillips, should be accorded greater evidentiary weight than the Examiner's unsuccessful search and unsubstantiated speculation as to the intended meaning of the questioned term.

In summary, when following the Phillips decision and reading the claim language within the context of the specification and with the understanding of a person skilled in the medical art, Applicant believes that there can be no real question as to the meaning of "multifactorial." The meaning of "non-specific" as being synonymous with "non-specialized" is apparent from previous submissions. Accordingly, the indefiniteness rejection should be withdrawn.

Claims 248, 249, and 252 stand rejected under 35 U.S.C. §112, first paragraph, as being based on an inadequate written description. The Examiner posits that the specification fails to provide a description of delivering cells via intraluminal injection, intravenous injection or angioplasty delivery of cells. The specification at page 45 describes injecting growth factors into a patient "intravenously, intraluminally, or intramuscularly to promote the growth of an artery" and

applying "genes or other genetic material – with an angioplasty balloon." The Examiner posited that the specification discloses that growth factors comprise cells but that there is no teaching that "other genetic material" describes a genus encompassing cells. Applicant disagrees with such position and has submitted evidence and arguments in the record establishing that such generic disclosure is sufficient to support claims drawn to a subgenus of cells. Capon v. Eshhar v. Dudas, 03-1480-1481, decided by the CAFC on August 12, 2005, is controlling precedent that Section 112 does not require recitation in the specification of features already known by workers in the technological field to which the invention is directed. The Examiner appears to have taken the position that generic inventions involving biochemical processes require a higher threshold for compliance with Section 112 because of a perception that success is not assured. However, it is clear from the Capon decision that generic biologic inventions are not rendered invalid simply because success of biochemical processes may not be assured. The Court in Capon further observed that the PTO must determine sufficiency of support on a case-by-case basis given the state of the pertinent art at the time of the invention and in light of the evidence of record with respect to the conceptuality of the invention and significance of the examples (written description) set forth in the specification. The Examiner has failed to address the generic concept that Applicant described – the concept of selecting a growth factor (herein the elected subgenus cells) and administering such growth factor into the body of a human patient using conventional methods and apparatus to grow cardiac muscle and an artery and repair a dead/damaged portion of the patient's heart, which would not naturally occur. The record does not show that this concept was known in the medical art prior to Applicant's invention and includes evidence of experimental verification as well as the potential viability in the concept. Perforce, the Examiner's reasoning that the specification is inadequate

because it does not specifically describe or set forth an example using each of these known delivery modes to deliver cells is inapt and should be withdrawn.

Applicant further submits that the Examiner's 35 U.S.C. §112 first paragraph rejection of (1) claims 248 and 249; and (2) claims 236, 238, 239, 243-253, and 256 on the grounds that one of skill in the art could not make and use the claimed invention without undue experimentation, citing In re Wands, must fail when considering the totality of the evidence in the record. Applicant's specification describes standard systems of identification as well as known procedures for selecting and isolating known cells (bone marrow stem cells) and known apparatus and methods for administering such cells to achieve the described desired therapeutic result. The specification describes/teaches specific materials and specific administration route for practicing the invention. Furthermore, the Examiner, in making this rejection, has failed to consider the generic concept described in the instant specification – the concept of selecting well-known appropriate cells and administering such cells using well-known methods and apparatus to grow muscle and arteries in a human patient heart that do not occur in nature. The Examiner has failed to cite any evidence showing this concept in the prior art. Rather, Applicant has submitted evidence in the record, including experimental verification as well as potential viability of the concept in the form of the autopsy report embodied in the Circulation publication entitled, "Transendocardial Autologous Bone Marrow Mononuclear Cell Injection in Ischemic Heart Failure," authored by Perin et al. and submitted as Exhibit A of the Supplement to Appellant's Appeal Brief, filed August 4, 2005. This publication is an updated report of the clinical trial reported in the Perin et al. Circulation publication in 2003 entitled, "Transendocardial, Autologous Bone Marrow Cell Transplantation for Severe, Chronic Ischemic Heart Failure" (attached hereto as Exhibit E). The Perin et al. trial used autologous bone marrow stem cells collected from the patients that were cultured and transplanted

into the patients using standard administrative techniques and standard apparatus resulting in the growth of new cardiac muscle and arteries in the patients. There is nothing in Perin et al., which explicitly or implicitly teaches or suggests that anything more than routine experimentation was required to carry out the implantation technique; rather it appears that Perin et al. followed the basic regimen described by Applicant. Perforce, based on this record, the Examiner's rejection of the claimed subject matter based on undue experimentation must fail.

In view of instant amendment and the other papers made of record by filing the RCE, Applicant respectfully believes that the application is in condition for allowance; and a notice to such effect is requested. Should the Examiner have any questions or wish to discuss any issues, a phone call to Applicant's attorney would be appreciated.

Respectfully submitted,

Dated: 11/16/05

Gerald K. White
Reg. No., 26,611

Dated: 11/16/05

Charles N. Lovell
Reg. No. 38,012

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Merriam-Webster

Medical Dictionary

83 entries found for **factor**. Select an entry and then click 'Go'.

factor
animal protein factor
antianemic factor
antihemophilic factor
atrial natriuretic factor
chill factor

Go

Main Entry: **fac·tor**
Pronunciation: ˈfak-tər
Function: *noun*
1 a : something that actively contributes to the production of a result b : a substance that functions in or promotes the function of a particular physiological process or bodily system
2 : GENE
- **fac·to·ri·al** /fak-ˈtōr-ē-əl, -ˈtȯr-/ *adjective*

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SEARCH

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Merriam-Webster

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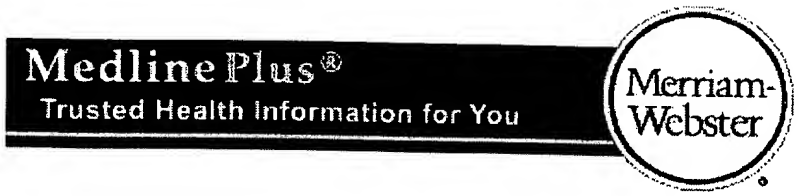
Pronunciation Key

\ ə \ as a in abut	\ g \ as g in go	\ r \ as r in red
\ ˈə ɪə \ as u in abut	\ h \ as h in hat	\ s \ as s in less>
\ ᵊ \ as e in kitten	\ i \ as i in hit	\ sh \ as sh in shy
\ ər \ as ur/er in further	\ ī \ as i in ice	\ t \ as t in tie
\ a \ as a in ash	\ j \ as j in job	\ th \ as th in thin
\ ā \ as a in ace	\ k \ as k in kin	\ th \ as th in the
\ ä \ as o in mop	\ k \ as ch in ich dien	\ ü \ as oo in loot
\ aů \ as ou in out	\ l \ as l in lily	\ ů \ as oo in foot
\ b \ as in baby	\ m \ as m in murmur	\ v \ as v in vivid
\ ch \ as ch in chin	\ n \ as n in own	\ w \ as w in away
\ d \ as d in did	\ ŋ \ as ng in sing	\ y \ as y in yet
\ e \ as e in bet	\ ō \ as o in go	\ yü \ as you in youth
\ ˈē ɪē \ as ea in easy	\ ȯ \ as aw in law	\ yů \ as u in curable
\ ē \ as y in easy	\ ȯi \ as oy in boy	\ z \ as z in zone
\ f \ as f in fifty	\ p \ as p in pepper	\ zh \ as si in vision



Medical Dictionary

One entry found for **multifactorial**.

Main Entry: **mul·ti·fac·to·ri·al**
Pronunciation: -fak-ˈtōr-ē-əl, -ˈtȯr-
Function: *adjective*
1 : having characters or a mode of inheritance dependent on a number of genes at different loci
2 *or* **mul·ti·fac·tor** /-ˈfak-tər/ : having, involving, or produced by a variety of elements or causes <a *multifactorial* study> <a disease with a *multifactorial* etiology>
- **mul·ti·fac·to·ri·al·ly** /-ē-ə-lē/ *adverb*
- **mul·ti·fac·to·ri·al·i·ty** /-ˌtōr-ē-ˈal-ət-ē, -ˌtȯr-/ *noun*, *plural* **-ties**

Search here for another word:

SEARCH [ ] Look it up

Pronunciation Key

\ ə \ as a in abut	\ g \ as g in go	\ r \ as r in red
\ ˈə ˌə \ as u in abut	\ h \ as h in hat	\ s \ as s in less>
\ ᵊ \ as e in kitten	\ i \ as i in hit	\ sh \ as sh in shy
\ ər \ as ur/er in further	\ ī \ as i in ice	\ t \ as t in tie
\ a \ as a in ash	\ j \ as j in job	\ th \ as th in thin
\ ā \ as a in ace	\ k \ as k in kin	\ th \ as th in the
\ ä \ as o in mop	\ k̲ \ as ch in ich dien	\ ü \ as oo in loot
\ aů \ as ou in out	\ l \ as l in lily	\ u̇ \ as oo in foot
\ b \ as in baby	\ m \ as m in murmur	\ v \ as v in vivid
\ ch \ as ch in chin	\ n \ as n in own	\ w \ as w in away
\ d \ as d in did	\ ŋ \ as ng in sing	\ y \ as y in yet
\ e \ as e in bet	\ ō \ as o in go	\ yü \ as you in youth
\ ˈē ˌē \ as ea in easy	\ ȯ \ as aw in law	\ yu̇ \ as u in curable
\ ē \ as y in easy	\ ȯi \ as oy in boy	\ z \ as z in zone
\ f \ as f in fifty	\ p \ as p in pepper	\ zh \ as si in vision

(*Circulation.* 2003;107:929.)

Mini-Review: Expert Opinions

Stem Cell Therapy in Perspective

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Introduction

The concept of regenerative medicine using the body's own stem cells and growth factors to repair tissues may become a reality as new basic science works and initial clinical experiences have "teamed-up" in an effort to develop alternative therapeutic strategies to treat the diseased myocardium. In particular, revealing

the signals that mediate cellular growth and differentiation may provide novel tools designed for myocardial regeneration in patients sustaining ischemic cardiomyopathy syndromes. We attempt herein to provide a critical overview of recent developments of myocardial cell transplantation strategies.

Stem Cells

Stem cells are a population of immature tissue precursor cells capable of self-renewal and provision of de novo and/or replacement cells for many tissues. Embryonic stem cells can be obtained from the inner cell mass of the embryonal blastocyst. Although it was recently shown that human embryonic stem cells can differentiate into cardiomyocytes,[1] because of the immunogenicity and rejection, as well as ethical considerations, these cells may be restricted to experimental in vitro studies and their therapeutical potential remains to be determined. Also, these cells may act as an unanticipated arrhythmogenic source after intramyocardial transplantation.[2] Clinical application of these cells is most likely years ahead (Table).

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Advantages and Disadvantages of Embryonic Versus Adult Stem Cells

In contrast, adult human stem cells (hematopoietic, mesenchymal) are found in mature tissues, eg, the bone marrow. Plasticity of adult stem cells can probably generate lineages of cells different from their original organ of origin. Thus, these cells can be used for organ regeneration and for cellular repair in various species, as well as in humans.

Ethical problems for adult autologous stem cells do not exist, and although much experimental work remains to be done, their clinical relevance and therapeutic benefit in heart disease have recently been shown for the first time.[3]

Except for hematopoietic and mesenchymal stem cells, many other bone marrow-related cell types may participate in organ repair of infarction models; bone marrow hemangioblasts take part in neovascularization, mesodermal progenitor cells are contained within the mononuclear bone marrow cell fraction that differentiates to endothelial cells, and endothelial progenitor cells can transdifferentiate into cardiomyocytes. Primitive bone marrow cells mobilized by stem cell factor and granulocyte-colony stimulating factor are capable of homing to infarct regions, replicating, differentiating, and promoting myocardial repair.[4] Ultimately, a variety of different cell types from the mononuclear bone marrow cell

fraction contribute to the regeneration of necrotic myocardium and damaged vessels. In this regard, therapeutic use of mononuclear cell populations of bone marrow may be more useful and promising than single isolated cell fractions alone. The effect manifested by more heterogenous bone marrow cell populations that contain very small numbers of stem cells may also suggest the importance of an entire array of bone marrow-derived growth factors and cytokines that may also regulate cellular growth and regeneration via cellular secretion mechanisms.

Stem Cells and Angiogenesis

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The complex cellular and molecular mechanisms by which endothelial and smooth-muscle cells interact with each other to form blood vessels are now better understood.[5] Endothelial cells alone can initiate the formation and sprouting of endothelium-lined channels, namely angiogenesis, in response to a physiological or pathological stimulus. Peri-endothelial cells are required for vascular maturation. Recruitment of smooth muscle cells provides these vessels with essential viscoelastic and vasomotor properties and enables accommodating the changing needs in tissue perfusion. This later stage is called arteriogenesis and has a major role in collateral growth.[6] Endothelial progenitor cells could be isolated from peripheral blood and/or bone marrow and showed incorporation into sites of physiological and pathological neovascularization in vivo after either systemic injection or using direct intramyocardial transplantation.[7] In contrast to differentiated endothelial cells, transplantation of progenitor cells successfully enhanced vascular development by in situ differentiation and proliferation within ischemic organs.[8] On the basis of these findings, the beneficial property of endothelial progenitor cells is attractive for angiogenic cellular interventions and as cell-mediated vehicles for gene therapy applications targeting regeneration of ischemic tissue and of failing hearts.

Stem Cell Differentiation to Muscle Cells

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- Conclusions and Open Questions
- References

The principal aim is to transplant cells of primarily noncardiac origin, such as human bone marrow-derived mononuclear cells containing human stem cells. These cells may operate as a precursor of heart muscle tissue and of coronary blood vessel cells. Human bone marrow contains hematopoietic (1% to 2%) and mesenchymal stem cells (<0.05%). Both types of stem cells may contribute to heart muscle repair. Hematopoietic stem cells are progenitor cells for many types of cells, eg, endothelial cells, which may also differentiate to heart muscle cells. Mesenchymal stem cells are progenitor cells for types of cells such as heart muscle cells, as well as for a variety of cells of noncardiac concern. Recent results in mouse experiments suggest the potency of extracardiac progenitor cells for transdifferentiation into new cardiomyocytes after acute experimental myocardial

infarction.[4] Bone marrow cells cultured with 5-azacytidine differentiated into cardiac-like muscle cells in culture and in vivo in ventricular scar tissue in pigs and improved myocardial function.[9] In clinical myocardial infarction, evidence has been provided that autologous bone marrow stem cells may regenerate in infarcted myocardium and improve myocardial perfusion of the infarct zone.[3] Studies with transplanted human hearts have shown that adult humans have extracardiac progenitor cells capable of migrating to and repopulating damaged myocardium, a process occurring at very low levels.[10] Recently, cases have been described in which a male patient receives a heart from a female donor, which provided an opportunity to test whether progenitor cells translocate from the recipient to the graft on the basis of Y chromosome labeling.[11] Results showed that myocytes, coronary arterioles, and capillaries that had a Y chromosome made up 7% to 10% of those in the donor hearts and were proliferative. This indicates a regenerative capacity of the transplanted myocardium. Thus, there is growing evidence for a repair function of extracardiac cells, eg, from bone marrow in the case of cardiac lesion and the necessity of myocardial healing, although these results are not unanimously approved.[12]

Milieu-Dependent Differentiation and Enhanced Environment

Studies from several species demonstrate that bone marrow-derived stem cells are stem cells for various mesenchymal tissues. The cells are therefore not simply stromal precursors, but precursors of peripheral tissues, such as heart muscle.[13] Normal growth and ultimate stem cell fate depend on engraftment in an appropriate "niche." Nonetheless, the mechanisms by which the local milieu influences stem cell differentiation are as yet undetermined. Thus, it seems that the fate of bone marrow stem cells is determined by the environment in which they engraft rather than by an intrinsically programmed fate. Therefore, enhancement of functional activity of the specific organ's niche for heart muscle, eg, by positive inotropic (pharmacologic augmentation of contractility) or by positive chronotropic stimuli (heart rate increase by exercise), may promote and intensify the transdifferentiation of bone marrow-derived stem cells to the cardiomyocyte phenotype. After an injury, eg, myocardial infarction, or a cellular damage, eg, in severe pressure or volume overload of the heart, specific factors, including cytokines, stem cell factor, and various growth factors, that stimulate cell replication and substitution in the injured tissue are released by the surrounding cells. In addition, transplanted stem cells, differentiating to cardiomyocytes, become indistinguishable over time from the surrounding cardiomyocytes, and they begin to express the contractile proteins specific for striated heart muscle, including desmin, a-myosin, heavy chain, a-actinin, and phospholamban at levels that are the same as in the host cardiomyocytes.[14] This transdifferentiation process is more pronounced in injured tissue than in healthy organs and may be intensified when the heart as the recipient organ contributes to its enhanced environment by high chronotropic and inotropic activity. Thus, regionally large concentrations of stem cells and increased mechanical activity of the recipient heart muscle may provide a favorable environment for successful engraftment of stem cells after cardiac injury.

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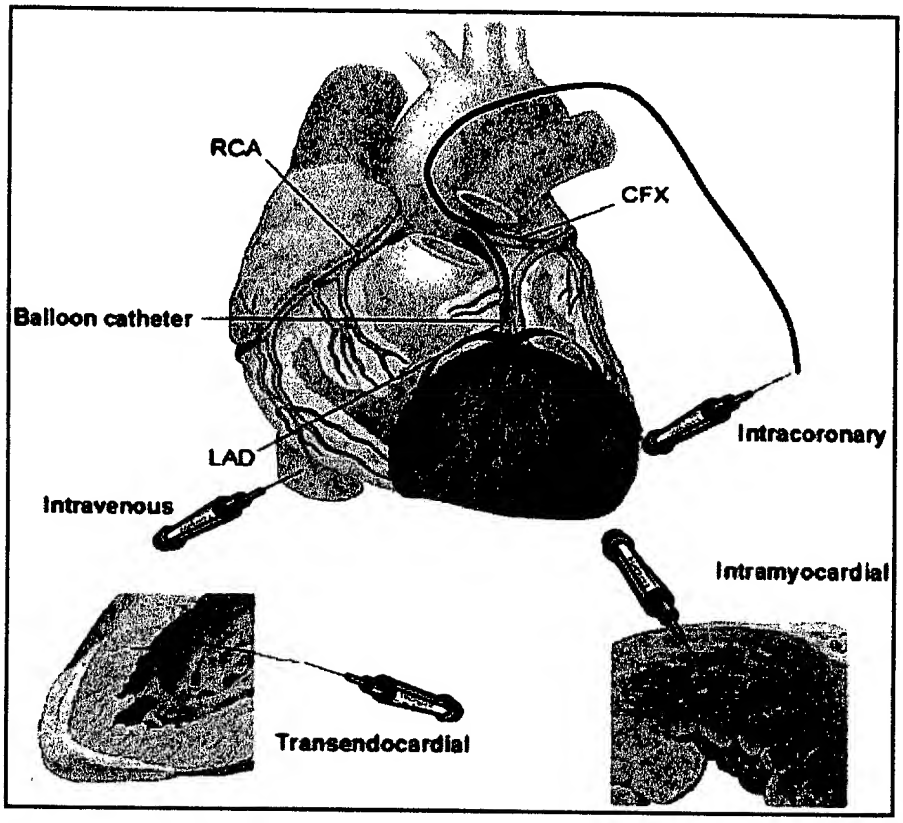
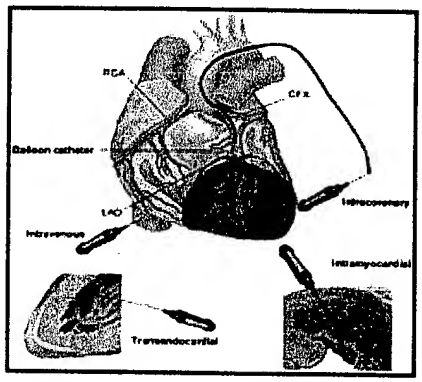


Delivery options for stem cell transfer modalities to the heart. The red colored area represents apical lesion of the left ventricle by myocardial infarction. The balloon catheter is localized in the infarct-related artery and is placed above the border zone of the infarction. Blue and green arrows suggest the possible route of cell infusion and migration into the infarct. The 2 small figures depict the transendocardial and intramyocardial route of administration. RCA indicates right coronary artery; LAD, left anterior descending coronary artery; and CFX, circumflex artery.

Route of Cell Administration

The appropriate route of cell administration to the damaged organ is an essential prerequisite for the success of organ repair (Figure). High cell concentrations within the area of interest and prevention of homing of transplanted cells into other organs are desirable. Therefore, targeted and regional administration and transplantation of cells should be preferred. Below, several special routes of administration are described.



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Delivery options for stem cell transfer modalities to the heart. The red colored area represents apical lesion of the left ventricle by myocardial infarction. The balloon catheter is localized in the infarct-related artery and is placed above the border zone of the infarction. Blue and green arrows suggest the possible route of cell infusion and migration into the infarct. The 2 small figures depict the transendocardial and intramyocardial route of administration. RCA indicates right coronary artery; LAD, left anterior descending coronary artery; and CFX, circumflex artery.

- In regional heart muscle disease, as in myocardial infarction, selective cell delivery by intracoronary catheterization techniques leads to an effective accumulation and concentration of cells within the infarcted zone. This can be realized in humans with bone marrow-derived cells.[15] With intracoronary administration, all cells must pass the infarct and peri-infarct tissue during the immediate first passage. Accordingly, with the intracoronary procedure, the infarct tissue can be enriched with the maximum available number of cells at all times. Further developments of catheterization systems for various clinical studies are needed.
- The transendocardial and transpericardial route of application has been used in large animal experiments[16] and was also recently tested in patients.[17] The main potential advantage of the surgical procedure is injection under visualization, which allows anatomic identification of the target area and even distribution of the injections. The safety and feasibility of catheter-based transendocardial injection was demonstrated in large animal studies,[18] and initial clinical experience in 19 patients using intramyocardial gene transfer showed similar safety profiles.[19] Current clinical experience is limited to one injection system, using electromechanical mapping to generate 3-dimensional left ventricular reconstruction before the injection. Intraventricular catheter manipulation, however, can injure the myocardium, inducing ventricular premature beats and short runs of ventricular tachycardia. In certain cases, this precludes injection to the more

arrhythmogenic zones, and it may extend the duration of the procedure and should always be carefully monitored. Each injection catheter is tested for cell biocompatibility to assure no mechanical or functional damage to cells being propelled under pressure through the narrow injection needle. Future developments with steerable transendocardial injection and delivery systems with mapping of the injured zone are needed. Transendocardial injection of autologous bone marrow cells has also been performed as part of several pilot and phase I studies. Safety and feasibility data are still pending and efficacy parameters need large randomized clinical trials.

- The intravenous route of administration is easiest. The main disadvantage, however, is that approximately only 3% of normal cardiac output will flow per minute through the left ventricle, and it is also limited because of transpulmonary first-pass attenuation effect on the cells. Therefore, this administration technique will require many circulation passages to enable infused cells to come into contact with the infarct-related artery. During that time, homing of infused cells to other organs will considerably reduce the number of cells that will populate the infarcted area.
- Some major cell types, such as skeletal myoblasts, have the disadvantage of an emboligenic potency when delivered systemically. Therefore, intramyocardial injection during open-heart surgery has been tested. This procedure has also been used in humans.[20] However, the therapeutic effect is limited because of severe arrhythmogenic complications. Another approach implanted autologous bone marrow cells during open-heart surgery and could show improvement in myocardial perfusion in 3 of 5 treated patients.[21]

Detection of Transplanted Stem Cells

An important clinical problem will be the identification and localization of transplanted autologous stem cells within the injured area of the heart. The transplanted cell or cell population is a single unit in a complex biological network of other cells. Therefore, for both localization and fate mapping of stem cells within the target organ, specific cell markers are desirable. Thus, analysis of stem cell behavior will presume (1) in situ labeling of a single cell or a transplanted cell population or (2) transplantation of already in vitro labeled cells or cell populations. For labeling in animal experiments, retroviral transduction with a marker gene or labeling with thymidine or bromodeoxyuridine (BrdU) have been used. For clinical detection of stem cells, magnetic labeling and in vivo tracking of bone marrow cells by the use of magnetodendrimers or radioactive detection methods may be useful. Myocardial biopsies in humans hardly will be justifiable under these circumstances. Thus, localization and fate mapping of stem cells in the region of myocardial injury will represent an important task for experimental and clinical stem cell research in the future, as well as for the assessment of time course of proliferation in the recipient new cell homes and for the evaluation of proper cell function after full transdifferentiation. First results through the detection of the reporter gene *LacZ*, by identification of ß-galactosidase–positive cells in tissue section and chromosome analysis by fluorescence in situ hybridization (FISH) techniques are encouraging.[22]

Stem Cells for Cardiac Wound Repair: A Joint Clinical and Experimental Approach

In the regenerating tissues, stem cells and progenitor cells in the microenvironment both take part in the renewal process. Bone marrow cells injected or mobilized to the damaged myocardium were shown to behave as cardiac stem cells with remarkable plasticity, giving rise to myocytes, endothelial cells, and smooth muscle cells.[23] In the case of human infarcted tissue, autologous bone marrow cells have shown to be highly effective in wound repair in terms of regenerating heart muscle and improving perfusion in the infarcted and border zone area.[24,25] Clinical studies therefore are necessary — in parallel to basic and experimental investigations — analyzing the promising prerequisites for clinical wound repair, preferably the optimum cell administration to the region of interest of the heart, eg, the infarcted tissue, and their optimum concentration and accumulation by different catheter-based techniques.

Moreover, catheter-guided cell transfer to the human heart has the unique advantages of being safe under local anesthesia and during routine cardiac catheterization, being fast, taking between 20 to 40 minutes for the whole procedure, and allowing the administration of bone marrow cells in abundance, selected or non-selected, from bone marrow puncture to the region of interest, which permits a much greater availability of stem cells for the heart than the normal wound healing in various heart diseases or in cardiac transplantation models per se would bring about.[15]

Experimental studies will be needed simultaneously to differentiate between the therapeutically most successful kinds of bone marrow cells:

Global bone marrow containing all mononuclear bone marrow cells or specifically selected subfractions, as isolated cell fractions containing preferably CD34+ or CD34-, CD45-, or AC133+ cells.

Analysis of the transdifferentiation of bone marrow cells to muscle cells and their contribution to the remodeling process in various heart diseases, including cardiac transplantation models.

Cardiac lesions may be multifactorial and include myocardial infarction, myocarditis, cardiomyopathy or cardiac remodeling due to severe pressure, and volume overload. It is uncertain whether the same therapeutic approach and the same type of cells will be suitable for all of these different diseases. However, organ repair by stem cells represents a general biological mechanism. Thus, it will be one of the future tasks to find the most practical and specific way of evolving and targeting the healing potency of stem cells for selected cardiovascular diseases.

Therapeutic Alternatives in Advanced Heart Failure

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Except for pharmacotherapeutics and other measures, the therapy of severe global heart failure and of advanced regional contraction insufficiency is based on nonpharmacological interventions. These are aimed at unloading the heart (cardiac assist device), harmonizing the electrical and mechanical course of contraction and relaxation (ventricular synchronization), restoring ventricular geometry by ventricular size diminution (myocardial left ventricular resection), or abolishing detrimental volume overload in mitral incompetence (repair of the mitral valve).[26,27] The clinical limitations of all of these approaches, which are aimed at reducing systolic wall stress and myocardial oxygen consumption,[28] justify the search for alternative therapeutic options that may beneficially modify the natural course of the disease. By stem cell-derived de novo restoration of damaged cells, replacement of destroyed and scarred tissue with the consecutive improvement of ventricular performance may be possible. It may be speculated that future therapeutical options of combined therapeutical strategies, eg, ventricular resynchronization together with myocardial stem cell repair, may result in additive therapeutical benefit.

Conclusions and Open Questions

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Stem cell therapy represents a fascinating new approach for the management of heart diseases. Recent clinical results have shown the feasibility of adult autologous cell therapy in acute myocardial infarction in humans. However, many unresolved questions about experimental and clinical cardiology are still open for future research, especially many basic problems concerning, among others, the following issues:

- The long-term fate of transplanted stem cells in the recipient tissue.
- The ability of transplanted stem cells to find their optimum myocardial "niche."
- The potency of stem cells to transdifferentiate into heart muscle cells.
- The optimal angiogenic milieu needed for transplanted cells in hypoperfused tissue.
- The capability of the recipient tissue to enable an enhanced environment to offer optimum, milieu-dependent differentiation of engrafted cells.
- Specific detection of engrafted cells or cell populations by labeling techniques.
- The optimal time course of availability and application for stem cell replacement therapy in cardiovascular disease.
- The arrhythmogenic potential of implanted cells.
- The specific characterization of the progenitor cells that should be measured to predict therapeutic effect of transplanted cells.
- Development of safe and reproducible catheter-based delivery systems for depositing stem cells to

recipient heart muscle.

Additional research is needed o explore the therapeutic merits of cell transplantation techniques while accepting the likelihood that possible adverse side effects may occur. With regard to the clinical practicability, ethical problems, and hazards of immunogenity, actual and future research will focus preferably on adult stem cells, whereas research on embryonic stem cells may emerge presumably into comparable clinical relevance in several years to come.

Footnotes

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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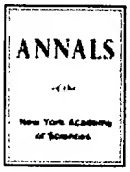

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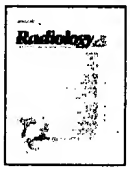

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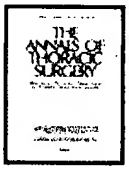

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Regeneration of Human Infarcted Heart Muscle by Intracoronary Autologous Bone Marrow Cell Transplantation in Chronic Coronary Artery Disease

The IACT Study

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OBJECTIVES Stem cell therapy may be useful in chronic myocardial infarction (MI); this is conceivable, but not yet demonstrated in humans.

BACKGROUND After acute MI, bone marrow-derived cells improve cardiac function.

METHODS We treated 18 consecutive patients with chronic MI (5 months to 8.5 years old) by the intracoronary transplantation of autologous bone marrow mononuclear cells and compared them with a representative control group without cell therapy.

RESULTS After three months, in the transplantation group, infarct size was reduced by 30% and global left ventricular ejection fraction (+15%) and infarction wall movement velocity (+57%) increased significantly, whereas in the control group no significant changes were observed in infarct size, left ventricular ejection fraction, or wall movement velocity of infarcted area. Percutaneous transluminal coronary angioplasty alone had no effect on left ventricular function. After bone marrow cell transplantation, there was an improvement of maximum oxygen uptake (Vo_{2max}, +11%) and of regional ^{18}F-fluor-desoxy-glucose uptake into infarct tissue (+15%).

CONCLUSIONS These results demonstrate that functional and metabolic regeneration of infarcted and chronically avital tissue can be realized in humans by bone marrow mononuclear cell transplantation. (J Am Coll Cardiol 2005;46:1651–8) © 2005 by the American College of Cardiology Foundation

Cardiac performance after myocardial infarction (MI) is compromised by ventricular remodeling, which represents a major cause of late infarct-related chronic heart failure and death (1,2). Although conventional drug therapy (e.g., with beta-receptor blockers and/or angiotensin-converting enzyme inhibitors) may delay remodeling, there is no basic

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therapeutic regimen available for preventing or even reversing this process. By the use of interventional therapeutics (percutaneous transluminal coronary angioplasty [PTCA], stent), recanalization of the occluded infarct-related artery is possible, thereby improving or normalizing coronary blood flow. However, despite sufficient reperfusion of infarcted tissue, the viability of the infarcted myocardium cannot, or can only insufficiently, be improved in most of these patients (3). Therefore, catheter-based therapy of acute MI is useful for vascular recanalization, but the second and crucial step, the regeneration of necrotic heart muscle, is not realized by this vascular procedure alone.

Experimental (4) and clinical (5,6) studies have shown recently for the first time that bone marrow mononuclear cells (BMCs) may regenerate damaged myocardium in acute MI in humans. Because the regenerative potential of bone marrow-derived cells ought also to be expected to exist in chronically ischemic heart disease as well (7–12), we have assembled in an ongoing clinical investigation 18 patients with chronic MI to prove this new therapeutic possibility.

METHODS

Study population. All 18 patients (49 ± 11 years) were men and were recruited consecutively from January 2003 until March 2004. They had had transmural MI 27 ± 31 months before, at which point all infarcts had been treated acutely by PTCA and/or stent implantation (Table 1, Fig. 1).

The inclusion criteria were age <70 years, one-vessel disease with an open infarct-related artery at the time of stem cell therapy, sinus rhythm, a clear-cut demarcation of the ventriculographic infarct area, and no coronary bypass surgery. General exclusion criteria were severe comorbidity and alcohol or drug dependency. Although chronically infarcted myocardium usually does not regenerate sponta-

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Abbreviations and Acronyms

BMC	= bone marrow mononuclear cell
CPK	= creatine phosphokinase
ECG	= electrocardiogram
LV	= left ventricular
MI	= myocardial infarction
PET	= positron emission tomography
PTCA	= percutaneous transluminal coronary angioplasty
Tx group	= transplantation group

neously, for comparison a control group, parallel to the recruitment of the stem cell transplantation group (Tx group), was recruited and analyzed, meeting the same inclusion criteria as the stem-cell group. The recruitment of patients was performed according to a randomization procedure in which all patients of the entire chronic infarction group were distributed to the treatment group, where they agreed with all the therapeutic regimen. Alternatively, all patients of the chronic infarction group who refused the therapeutic regimen (bone marrow puncture and aspiration, intracoronary cell transplantation, and another cardiac catheterization) were allocated to the control group. All medications with angiotensin-converting enzyme inhibitors and with beta receptor blockers were maintained constant during the study period.

The cell-treated patients had stable ventricular dynamics for infarct size, ejection fraction, and wall movement velocity of infarcted area at least 9 ± 6 months before cell transplantation. Infarct size at the time of cell therapy showed an amount of 27 ± 8% of the circumference of the left ventricle (LV), determined by ventriculography.

Preparation of BMCs. One day before cell therapy, bone marrow was taken (80 ml from the iliac crest) and mononuclear cells were isolated and identified including CD34-positive cells, AC133-positive cells and CD45/CD14 negative cells (6). The cells were isolated under good manufacturing practice conditions by Ficoll density separation on Lymphocyte Separation Medium (Bio Whittaker, Walkersville, Maryland), before the residual erythrocytes were lysed with H_2O. For overnight cultivation, 1×10^6 BMCs/ml were placed in Teflon bags (Vuelife, Cell Genix, Gaithersburg, Maryland) and cultivated in X-Vivo 15 Medium (Bio Whittaker) supplemented with 2% heat-inactivated autologous plasma. The next day, BMCs were harvested and washed three times with heparinized saline before final resuspension in heparinized saline. Viability was 93 ± 3%. Heparinization and filtration (cell strainer, FALCON) was carried out to prevent cell clotting and microembolization during intracoronary transplantation. These cells were used for therapy. All microbiologic tests of the clinically used cell preparations proved negative. All patients received extensive information about the procedure, which was approved by the ethical committee of our university, and all gave written informed consent.

Administration of BMCs. Following assessment of baseline examinations (coronary angiography, left ventriculography, spiroergometry, ^{99m}Tc-tetrofosmin single-photon emission computed tomography (SPECT) and ^{18}F-fluor-deoxy-glucose (^{18}F-FDG) positron emission tomography (PET), cell transplantation was performed via the intracoronary administration route (6,13) using four to six fractional infusions parallel to balloon inflation over 2 to 4 min of 3 to 5 ml of cell suspension, each containing 15 to 22 $\times$ 10^6 mononuclear cells. All cells were infused directly into the infarcted zone through the infarct-related artery via an angioplasty balloon catheter, which was inflated at a low pressure (2 to 4 atm) and was located within

Table 1. Demographic Data of Intracoronary Bone Marrow Stem Cell Transplantation Group and Control Group

Characteristics	Tx Group	Control Group	p
No. of patients	18	18	
Age, yrs	49 ± 11	52 ± 10	NS
Transmural myocardial infarction, months before Tx	27 ± 31	30 ± 34	NS
Coronary angiography			
LAD/LCX/RCA as affected vessel	16/0/2	10/3/5	
No. of patients with stent implantation	16	17	NS
Risk factors			
Diabetes mellitus, %	16	11	NS
Positive family history, %	44	33	NS
Smoker and ex-smoker, %	67	56	NS
Hyperlipoproteinemia, %	89	94	NS
Medication			
Beta-blocker, %	94	89	NS
Angiotensin-converting enzyme inhibitor, %	94	89	NS
Statin, %	94	100	NS
Laboratory parameters			
CPK, U/l	1,504 ± 979	1,489 ± 952	NS
Bone marrow mononuclear cells, n (10^6×)	90		

Values are mean ± SD or number of patients.
CPK = creatine phosphokinase; LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery; RCA = right coronary artery; Tx = intracoronary bone marrow stem cell transplantation.

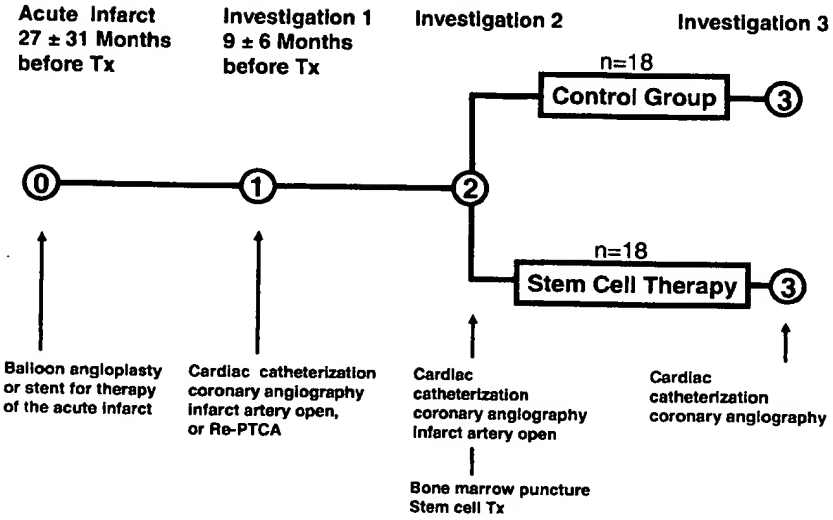

Figure 1. Diagrammatic representation of the algorithm of intracoronary stem cell therapy (Tx) in chronic ischemic heart disease after myocardial infarction. The infarcts occurred 27 ± 31 months before Tx. All infarct patients were treated with percutaneous transluminal coronary angioplasty (PTCA) or with stent implantation. 9 ± 6 months before (investigation 1) coronary angiography (including quantitative left ventriuclography) was performed. If re-stenosis was present, re-PTCA was made. Investigation 2 embraces all patients for the evaluation of coronary morphology after PTCA/stent. Only patients with an open infarct-related artery were included in both groups. Patients who agreed to Tx received within 10 days after investigation 2 bone marrow punctures and Tx by the intracoronary administration route and had altogether five invasive investigations, including two for therapeutic reasons (nos. 0 and 1). Patients who were not eligible for Tx (disagreement with bone marrow puncture and with subsequent Tx) served as a control group. Investigation 3 represents all follow-up measurements 3 months after Tx (Tx patients) or after investigation 2 for control group patients.

the previously stented coronary segments. This prevented backflow of cells and produced stop flow beyond the site of balloon inflation to facilitate high-pressure infiltration of cells into the infarcted zone. Prolonged contact time for cellular migration was also enabled. Three months after catheter-guided cell transplantation, all functional tests were repeated, including coronary angiography and left ventriculography. There were no procedural or cell-induced complications, and there were no side effects in any patient.

Spiroergometry. Aerobic exercise capacity was examined before (<10 days) intracoronary cell transplantation and three months later during follow-up. All patients (n = 18) were subjected to initial bicycle spiroergometry to assess their functional fitness and to determine the limit of safe intensity of exercise. We chose a protocol with an intensified workload up to the symptom-limited maximum (basic load of 50 W, intensification at 25 W, 2-min duration of each workload step). We determined the anaerobic threshold for prescribing a suitable load intensity. During the whole spiroergometry, monitoring by a 12-lead electrocardiogram (ECG) was carried out. The exercise capacity was assessed on the basis of maximum load levels expressed in watts (W_{max}) and maximum peak oxygen uptake (Vo_{2max}).

Coronary angiography and left ventriculography. Coronary angiography and biplane left ventriculography were performed 9 ± 6 months before cell transplantation and also a second time, within 10 days, immediately before cell therapy. The therapeutic follow-up was three months after the treatment. Thus, stable baseline conditions were documented (coronary vessel involvement, ventricular function, and geometry). Cardiac function was evaluated by left ventricular (LV) ejection fraction and by auxotonic myocardial contractility index, evaluated by the wall movement velocity of the infarcted area. The infarct size was calculated according to the method of Sheehan (14) by plotting five axes perpendicular to the long axis of the heart in the main akinetic or dyskinetic segment of the ventricular wall. Systolic and diastolic lengths were then measured by two independent observers, and the mean difference was divided by the systolic duration in seconds.

Quantification of coronary stenosis (restenosis). Cinecoronarangiograms were obtained during stem cell transplantation and at three months thereafter according to standard acquisition guidelines. The angiograms were evaluated by two independent observers and quantitative analysis was performed (15). Standard morphologic criteria were used to characterize the complexity of baseline lesions. The user-defined reference diameter proximal to the stenosis and the minimal luminal diameter within the culprit of the stenosis were used to calculate the percentage of stenosis. A value of 0 mm was assigned for the minimal luminal diameter in case of total occlusion at baseline or follow-up. Restenosis was defined as ≥50% stenosis of the initial target lesion at follow-up. Calculations of restenosis were performed in both groups, with and without stem cell therapy, in the same way, thus enabling evaluation the differential effects of PTCA-guided cell therapy and of PTCA effects alone.

Ventricular function after PTCA in the control group. For the evaluation of a potential effect on the PTCA intervention itself on LV function, all patients in the control group were analyzed with regard to infarct size, ejection fraction, and infarction wall movement velocity.

Table 2. Single Values of Intracoronary Bone Marrow Stem Cell Transplantation Group

Patient Number	Area of Infarction, %*			LV Ejection Fraction, %*			Infarction Wall Movement Velocity, cm/s*		
	Investigation 1 9 ± 6 Mo Before Tx	Investigation 2 <10 Days Before Tx	Investigation 3 3 Mo After Tx	Investigation 1 9 ± 6 Mo Before Tx	Investigation 2 <10 Days Before Tx	Investigation 3 3 Mo After Tx	Investigation 1 9 ± 6 Mo Before Tx	Investigation 2 <10 Days Before Tx	Investigation 3 3 Mo After Tx
1	26	26	22	56	55	60	0.88	0.77	0.82
2	28	29	26	45	43	49	2.06	1.88	2.13
3	16	16	5	64	65	71	1.45	1.50	2.10
4	27	25	14	48	50	65	1.20	1.25	2.88
5	16	14	11	66	69	71	2.25	2.77	3.75
6	16	13	6	64	66	73	1.50	1.77	2.55
7	15	18	11	57	55	63	2.78	2.65	3.13
8	28	28	20	43	44	49	3.15	3.25	4.25
9	27	27	11	46	46	64	1.61	1.65	3.30
10	20	17	14	56	58	62	2.21	2.45	3.13
11	28	25	17	42	38	52	1.91	1.88	3.00
12	33	28	21	44	47	54	2.28	2.62	3.50
13	39	37	27	50	51	59	1.25	2.50	4.90
14	29	33	27	62	62	61	1.20	1.33	2.70
15	37	37	31	48	43	53	1.83	1.56	2.50
16	29	29	24	53	54	58	1.25	1.06	3.06
17		41	35		48	55		1.66	3.00
18		35	25		45	53		0.94	1.94
Mean	26	27	19	53	52	60	1.80	1.86	2.92
SD	7	8	9	8	9	7	0.63	0.70	0.91

*Calculated from left ventriculography.
LV = left ventricular; Mo = Months; other abbreviations as in Table 1.

Nuclear cardiologic investigations (PET and SPECT). ^{18}F-FDG-positron emission tomography (^{18}F-FDG PET) was performed with a Scanditronix SCX 4096 WB-Scanner (FWHM = 6 mm transaxial, axial field of view = 4.6 cm). Patients received an oral glucose load of 1 g/kg body weight 80 ± 30 min before the intravenous application of ^{18}F-FDG (380 ± 60 MBq). The ^{18}F-FDG was administered at the time of decrease of blood glucose level <130 mg/dl. An initial transmission scan was obtained using a ^{68}Ga-filled pin source to correct the subsequent emission scans for attenuation. The data acquisition was started 45 min after administration of FDG. Image data were recorded with a 256 × 256 matrix in 3 consecutive bed positions over 15 min per position. The data were reconstructed back-projected with a Hanning filter (5 mm).

^{99m}Tc-tetrofosmin SPECT. Sixty minutes after intravenous injection of 600 ± 140 MBq of the perfusion-marker ^{99m}Tc-tetrofosmin under a "rest" condition, the images were obtained using a SPECT scanner with double-head detector (PRISM 2000, Marconi/Phillips), a low-energy, high-resolution collimator, and a 128 × 128 matrix. Image data were collected over 360° at 3° every 30 s. The images were reconstructed backprojected with a low-pass filter (order 12, cutoff 0.2).

PET and SPECT evaluation. Normalized values for FDG uptake and perfusion were calculated by comparing regional with maximum tracer uptake on the reconstructed images. We performed a regional analysis of glucose metabolism and perfusion using a set of standardized, individually adjusted circular regions of interest (diameter 18.06 mm, surface 256 mm^2). The reconstructed metabolic and perfusion images were realigned for each patient (MPI-Tool, version 3.0; Advanced Tomo Vision, Erftstadt, Germany) and were resliced according to cardiac axis (short-axis and horizontal and vertical long-axis views). The regions were positioned immediately neighboring, with no overlap, according to an overlay of the co-registrated metabolic and perfusion images. The regions covered the infarct lesion as well as normal myocardium. In this way, we generated templates of regions for each patient, which could be used for the evaluation of metabolism and perfusion, before and after BMC transplantation without further modification. According to Segall et al. (16), regions with a normalized FDG uptake <50% were rated as transmural scar and regions with an uptake of 50% to 60% as non-transmural scar.

Further analysis was restricted to regions with FDG uptake <60% in the PET scans, pursuant to our intention to focus on the effects of BMC transplantation on scar tissue.

Safety parameters. To assess any inflammatory response and myocardial reaction after cell therapy, white blood cell count, the serum levels of C-reactive protein (CRP) and of creatine phosphokinase (CPK) were determined immediately before as well as after treatment. Additional analysis was done directly after transplantation and three months later: ECG at rest, 24-h Holter ECG, and echocardiography.

Statistical analysis. All data are presented as mean ± SD. Statistical significance was accepted when $p < 0.05$. Intra-individual comparison of variables of investigation 1 (9 ± 6 months before cell transplantation for Tx group, 9 ± 5 months before investigation 2 for control patients) and investigation 2 (<10 days before cell transplantation for Tx group, no transplantation for control patients) and of variables of investigation 2 and follow-up investigation 3 (3 months after cell therapy for Tx group, 8 ± 5 months after investigation 2 for control patients) was performed using Wilcoxon rank-sum test. The missing values (Table 2) were omitted and not calculated for statistical analysis. The p values (by analysis of variance) have been given for LV ejection fraction, area of infarction, and infarction wall movement velocity. Statistical analysis was performed with SPSS-Windows 10.1 software.

RESULTS

Three months after intracoronary cell therapy, the infarct size was reduced by 30%, whereas the global LV ejection fraction increased by 15% and regional infarct wall movement velocity by 57% (Tables 2 and 3). In parallel, the clinical performance improved (Table 4), as evidenced by a higher work load demonstrated by a 11% increase in maximum oxygen uptake (Vo_{2max}). SPECT investigation presented enhanced tetrofosmin uptake in the infarcted zone by 5%, and PET examination showed enhanced glucose uptake in the infarcted zone by 15%, demonstrating regeneration of formerly avital, chronically infarcted heart muscle (Fig. 2). An unchanged or even impaired LV function was not observed in any patient.

In the control group (18 patients with chronic MI, but without stem cell therapy) no significant changes were observed in infarct size, LV ejection fraction, or wall

Table 3. Cardiac Parameters in the Transplantation Group and in Control Group at the Three Investigation Time Points

	Area of Infarction, %			LV Ejection Fraction, %			Infarction Wall Movement Velocity, cm/s		
	Control Group	Tx Group	p Value*	Control Group	Tx Group	p Value*	Control Group	Tx Group	p Value*
Investigation 1	25 ± 9	26 ± 7	0.99	53 ± 10	53 ± 8	0.87	1.95 ± 0.66	1.80 ± 0.63	0.57
Investigation 2	27 ± 9	27 ± 8	0.83	51 ± 10	52 ± 9	1.00	1.88 ± 0.76	1.86 ± 0.70	0.94
Investigation 3	26 ± 9	19 ± 9	0.02	52 ± 10	60 ± 7	0.02	1.91 ± 0.79	2.92 ± 0.91	0.001

*Analysis of variance.
Abbreviations as in Table 1.

Table 4. Positron Emission Tomography and Spiroergometry Before and After Stem Cell Therapy in Chronically Infarcted Myocardium

	^{18}F-FDG-Positron Emission Tomography		VO_{2max}, Spiroergometry	
	FDG Uptake, %	Difference in %	ml/min	Difference in %
Investigation 1	none		none	
Investigation 2	43.8 ± 8.0	+ 15	1,602 ± 533	+ 11
Investigation 3	50.5 ± 11.6		1,776 ± 523	
p (Wilcoxon test)	0.012		0.0001	

^{18}F-FDG = ^{18}F-fluor-deoxy-glucose; VO_{2max} = maximum oxygen uptake.

movement velocity of the infarcted area (Figs. 3A to 3C). Electrocardiogram at rest and on exercise and 24-h Holter ECG revealed no rhythm disturbances at any time point. Only 1 patient (from 18 cell-treated patients, 6%) developed relevant restenosis due to quantitative angiographic criteria. The restenosis could be treated adequately by stent implantation. The other 17 patients showed good patency rates without restenosis after PCI and cell transplantation. They also revealed no alterations in LV function 8 ± 5 months after PTCA.

There was no inflammatory response or myocardial reaction (white blood cell count, CRP, CPK) after cell therapy, despite a moderate increase in CRP (before cell transplantation 0.58 ± 0.48 mg/dl, after cell transplantation 1.07 ± 0.73 U/l, p = 0.002), which is usual after bone marrow puncture and/or cardiac catheterization.

DISCUSSION

The results of these investigations demonstrate, for the first time, that the intracoronary transplantation of autologous bone marrow mononuclear cells may reduce infarct size and improve LV function as well as myocardial glucose uptake in chronic ischemic heart disease attributable to chronic MI (5 months to 8.5 years old). Infarct size decreased in all patients and cardiac performance (ejection fraction, wall movement velocity of infarcted area, maximum oxygen uptake, and exercise tolerance) and myocardial metabolism (FDG-PET) improved, all being between 11% and 57%. Furthermore, it is noteworthy that there were no complications immediately or three months after cell transplantation, especially that there was no cardiac arrhythmia and no signs of cardiac or systemic inflammation were present.

The effects of stem cell transplantation on infarct size, cardiac function, and contractility demonstrate significant improvement of these three parameters in the therapy group (before and after stem cell therapy) as well as in the comparison between the stem cell therapy group and the control group, thus giving evidence for a beneficial therapeutic effect of stem cell therapy on cardiac performance in chronic MI.

Patients in both the stem-cell group and the control group were recruited in parallel to each other and consecutively between January 2003 and March 2004. They all (n = 36) fulfilled the same inclusion criteria. Thus, representative patient characteristics were present for the stem cell group (n = 18) and the control group (n = 18) as well as in comparing both of them. Moreover, two subsequent investigations before stem cell transplantation have been performed for each patient: investigation 1 and 2 demonstrated the stability of LV dynamics before cell therapy (9 months respectively 10 days before transplantation) and investigation 3 compared the effects of stem cell therapy with the control group. The stable hemodynamics during the preceding 9 ± 6 months before stem-cell therapy and the stable hemodynamics within the control group at all three points of investigation underline the significant alterations of the left ventriculography-derived parameters investigated after stem cell transpantation.

The regenerative potential of bone-marrow–derived stem cells may be explained by any of four mechanisms: 1) direct cell differentiation from mononuclear cells to cardiac myocytes (17), 2) cytokine-induced growing and increase of residual viable myocytes, especially within the border zone of the infarcted area (18), 3) stimulation of intrinsic myocardial stem cells (endogenous stem cells) (19,20), and 4)

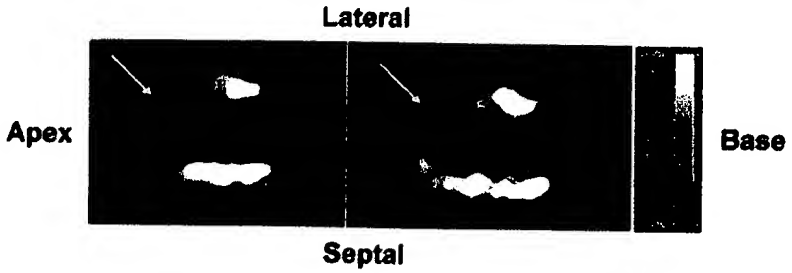

Figure 2. Representative illustration of ^{18}F-FDG-positron emission tomography (PET) before **(above)** and 3 months after **(below)** cell therapy in the transversal **(left)** and longitudinal **(right projection)** in a 30-year-old male patient with an 8-month-old anteroapical infarction. Note the restoration of glucose uptake **(below)** within the infarcted area of the formerly completely avital anteroapical myocardium.

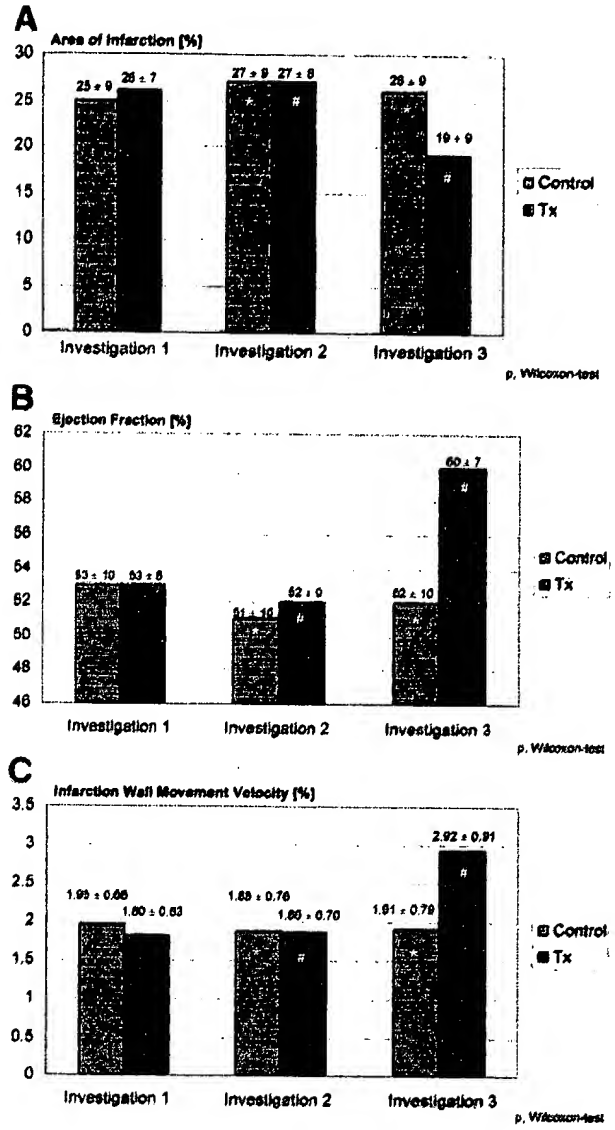

Figure 3. Illustration of the mean values of **(A)** area of infarction, **(B)** ejection fraction, and **(C)** infarction wall movement velocity, determined by quantitative left ventriculography in both groups (control group vs. transplantation [Tx] group) at the point of time: investigations 1, 2, and 3. Comparision of both groups with chronically infarcted myocardium (conrol group vs. Tx group), n = 18 patients. Investigation 1 was 9 ± 6 months before cell transplantation (controls: 9 ± 5 months before percutaneous transluminal coronary angioplasty [PTCA]); investigation 2 within 10 days before cell tranplantation (controls: at the time point of PTCA) and investigation 3 was three months after cell transplantation (controls: 8 ± 5 months after PTCA). Note the significant decrease of infarct size and the increase in ejection fraction and in contractility (infarction wall movement velocity) 3 months after cell therapy in comparison with the control group. *p = not significant (investigation 2 vs. investigation 3); #p = 0.001 (investigation 2 vs. investigation 3).

induction of cell fusion between transplanted bone marrow cells and resident myocytes (21–24).

Transdifferentiation has been described by previous investigators (4); however, it has been questioned by recent experimental studies (25). The influence of cytokines has shown to restore coronary blood vessels and muscle cells after experimental myocardial infarction. This regeneration of blood vessels and muscle cells is most pronounced in the border zone of ischemic and/or infarcted tissue (26), demonstrating an enhancement of mitotic cells and cell cycles up four-fold, when compared to areas remote from the necrotic myocardium. Moreover, mononuclear bone marrow stem cells contain a lot of cytokines (VEGF, insulin-like growth factor, platelet-derived growth factor, and so on), thereby stimulating residual normal myocytes for regeneration and proliferation and instrinsic myocardial stem cells (endogenous stem cells) for cell regeneration and for cell fusion (27–31).

Mitotic indexes are three to four times more frequent within the border zone of myocardial necrosis when compared with non-injured heart muscle (26). Moreover, 20% to 40% of intracoronarily transplanted bone-marrow–derived stem cells may be accumulated within the border zone of MI. There were no signs of apparent microcirculation disturbances because all patients had Thrombolysis In Myocardial Infarction flow grade 3. Thus, it is conceivable that in MI the border zone represents the optimum "niche" for exogenously transplanted stem cells, stimulating mitosis rates and heart muscle regeneration, preferably originating in and expanding from these areas. Cell fusion may also contribute to heart muscle regeneration, which takes its origin from the border zone, expanding gradually to the necrotic core of the infarcted area.

Our study cannot determine which cell-biologic and molecular mechanims are responsible for heart muscle repair or which of the studied factors may play the predominant role. However, the final functional outcome of this cell therapy demonstrates three main target effects: improvement in muscle function (pumping ability and contractility), myocardial perfusion (SPECT), and myocardial glucose metabolism (PET), thus giving evidence that heart muscle repair must have taken place by this intracoronary bone marrow cell transplantation procedure.

The clinical significance of this novel therapeutic approach may embrace a large number of patients with chronic coronary artery disease, preferably after previous or long-standing MI. It is conceivable that remodeling after infarction may be ameliorated or even stopped by this procedure. Thus, cell therapy may represent a new option of basic and causal therapy in chronic infarcted myocardium. It is an open question whether variations of the amount and kind of bone marrow cells, the administration technique, and the transplantation procedure itself, by enhanced environment and improvement of the angiogenic micromilieu, can further improve the milieu-dependent differentiation or regeneration of bone marrow cells in chronic infarcted heart disease. Therefore, our clinical results represent a stable basis to proceed to the next necessary step: to a larger prospective randomized study.

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Transendocardial, Autologous Bone Marrow Cell Transplantation for Severe, Chronic Ischemic Heart Failure.

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BACKGROUND: This study evaluated the hypothesis that transendocardial injections of autologous mononuclear bone marrow cells in patients with end-stage ischemic heart disease could safely promote neovascularization and improve perfusion and myocardial contractility. METHODS AND RESULTS: Twenty-one patients were enrolled in this prospective, nonrandomized, open-label study (first 14 patients, treatment; last 7 patients, control). Baseline evaluations included complete clinical and laboratory evaluations, exercise stress (ramp treadmill), 2D Doppler echocardiogram, single-photon emission computed tomography perfusion scan, and 24-hour Holter monitoring. Bone marrow mononuclear cells were harvested, isolated, washed, and resuspended in saline for injection by NOGA catheter (15 injections of 0.2 cc). Electromechanical mapping was used to identify viable myocardium (unipolar voltage >/=6.9 mV) for treatment. Treated and control patients underwent 2-month noninvasive follow-up, and treated patients alone underwent a 4-month invasive follow-up according to standard protocols and with the same procedures used as at baseline. Patient population demographics and exercise test variables did not differ significantly between the treatment and control groups; only serum creatinine and brain natriuretic peptide levels varied in laboratory evaluations at follow-up, being relatively higher in control patients. At 2 months, there was a significant reduction in total reversible defect and improvement in global left ventricular

function within the treatment group and between the treatment and control groups (P=0.02) on quantitative single-photon emission computed tomography analysis. At 4 months, there was improvement in ejection fraction from a baseline of 20% to 29% (P=0.003) and a reduction in end-systolic volume (P=0.03) in the treated patients. Electromechanical mapping revealed significant mechanical improvement of the injected segments (P<0.0005) at 4 months after treatment. CONCLUSIONS: Thus, the present study demonstrates the relative safety of intramyocardial injections of bone marrow-derived stem cells in humans with severe heart failure and the potential for improving myocardial blood flow with associated enhancement of regional and global left ventricular function.

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